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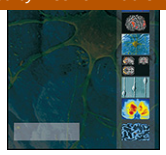

Dipyrone attenuates acute sickness response to lipopolysaccharide in mice

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ABSTRACT

Sickness behavior appears to be the expression of a central motivational state that reorganizes the organism's priorities to cope with infectious pathogens. To evaluate the effect of dipyrone in lipopolysaccharide (LPS)-induced sickness behavior, mice were subjected to the forced swim test (FST), tail suspension test (TST), dark–light box test, open field test, sucrose preference intake test and food intake test. LPS administration increased the immobility time in the TST, increased the time spent floating in the FST, and depressed locomotor activity in the open field test. Treatment with LPS decreased the total number of transitions made between the dark and light compartments of the apparatus and induced anhedonia and anorexia. Pre-treatment with dipyrone (10, 50, or 200 mg/kg) attenuated behavioral changes induced by LPS in the FST, TST, open field and light–dark box tests. In addition, dipyrone prevented anhedonia and anorexia in mice challenged with LPS. Considering that dipyrone attenuates LPS-induced behavioral changes, it is proposed that LPS-induced sickness behavior is dependent on the COX pathway.

1. Introduction

Sickness behavior is the expression of a motivational state triggered by an activation of the peripheral innate immune system and is characterized by depressive-like behavior, such as a reduction in locomotor activity and exploratory behavior, anorexia and anhedonia [7,17]. The mechanisms underlying sickness behavior have not been fully elucidated, but it has been suggested that cytokines and prostaglandins are involved [7,9,17,30]. Interleukin-1β, interleukin-6 and tumor necrosis factor-α (TNF-α) may be secreted in response to infections and endotoxemia [7,17]. Previous reports have demonstrated that LPS-induced depressive-like behavior appears to depend on the cyclooxygenase (COX) pathway as the use of a non-steroidal anti-inflammatory drug (NSAID) (indomethacin and nimesulide) has been shown to attenuate the behavioral changes induced by LPS [9]. COX is the key enzyme in the synthesis of prostaglandins from arachidonic acid. While COX-1 is a constitutive enzyme, COX-2 is induced by several stimuli, and the biosynthesis of both is inhibited by NSAIDs [31]. The pyrazolone derivative dipyrone, also known as metamizole, is an NSAID that acts as an effective analgesic and antipyretic and has been demonstrated to inhibit COX [25].

In contrast to classical NSAIDs, dipyrone produces analgesic effects associated with a less potent anti-inflammatory action in different animal models [15]. There are four major metabolites of dipyrone, but only the main metabolites, 4-methylamino-antipyrine (MAA) and 4-amino-antipyrine (AA), alter the biochemical properties of COX [25]. Considering the difference in the mechanism of action between dipyrone and the nonsteroidal anti-inflammatory drugs and the popularity of dipyrone as an analgesic and antipyretic drug during infective illnesses in many countries, the aim of the present study was to investigate the effects of pre-treatment with dipyrone on LPS-induced sickness behavior.

2. Materials and methods

2.1. Animals

Adult male Swiss mice (25–30 g), obtained from the Central Facility of the Federal University of Alfenas, were individually housed, for at least a week, in a room controlled temperature (24 ± 1 °C), humidity (40–60%) and a 12:12 h light–dark cycle (lights off at 6:00 pm). Standard rodent chow and tap water were provided *ad libitum* throughout the experiments except where indicated. Different animals were used for each experiment. Immediately after the end of the experiments, the animals were euthanized with halothane overdose so as to avoid any suffering.

All procedures were conducted in accordance with the Declaration of Helsinki on the welfare of experimental animals and with the approval of the Ethics Committee of the Federal University of

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Alfenas-MG (protocol number: 167/2008). All behavioral tests were conducted by an experimenter blind to the treatment groups.

2.2. Depressive-like and exploratory behavior

Mice were pre-treated with dipyrone (10, 50 or 200 mg/kg, i.p.) or vehicle (sterile saline, 0.9% NaCl, 1 ml/kg) 30 min before injection of LPS extracted from *Escherichia coli* serotype 026:B6 (200 μg/kg, i.p.; at 12:00 pm) or sterile saline (0.9% NaCl). The behavioral tests included the forced swimming test (FST), tail suspension test (TST), light–dark box test and open field test all of which were performed 120 min after LPS administration (for details, see Ref. [9]). The experiments were recorded using a video camera.

2.2.1. Forced swimming test (FST)

Mice ($n = 12$ per group) were placed in a vertical glass cylinder (26 cm high, 12 cm in diameter) filled with 25 °C water to a depth of 16 cm. For testing, each mouse was placed in the cylinder for 6 min and the duration of floating (i.e., the time during which mice made only the smallest movements necessary to keep their heads above water) was scored [9] from the film.

2.2.2. Tail suspension test (TST)

The mice ($n = 8$ per group) were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6 min period. The procedure was modified from Dunn and Swiergiel [10].

2.2.3. Light–dark box test

The apparatus consisted of a rectangular Plexiglas box (48 cm long × 24 cm wide × 24 cm high) divided into a dark and light region (both 24 cm long). The light and dark regions were separated by an opening (8 cm × 8 cm) that allowed the animals to move between the two compartments. The dark region was made of black Plexiglas and covered with a black lid. The light portion was made of white Plexiglas with a 60 W light positioned directly over it. The mice ($n = 10$ per group) were placed in the light compartment and allowed to move freely between the two compartments. Behavior was recorded for a total of 5 min and scored for latency to the first transition and the number of transitions between the light and dark compartments [9].

2.2.4. Open field behavioral test

Locomotor activity was recorded for 5 min in an open field consisting of a 60 cm × 60 cm white Plexiglas box with its floor divided into 16 squares. Four squares were defined as the center, and the 12 squares along the walls were considered the periphery. Each mouse ($n = 10$ per group) was gently placed exactly in the center of the box, and activity was scored as a line crossing when a mouse removed all four paws from one square and entered another. Line crossings among the central four squares or among the peripheral 12 squares of the open field were counted separately [9,10].

2.3. Sucrose preference intake test

Animals received food and water ad libitum and had access to 10% sucrose for 2 h every day (2:00–4:00 pm) for five days (sucrose intake training). After this period, to establish LPS doses that could produce a pronounced anhedonic behavior, doses of LPS (200, 500 or 1000 μg/kg, i.p.; $n = 10$ animals per group) or saline were injected at 12:00 pm. At 2:00 pm, mice had access to water and 10% sucrose for 24 h. This experiment was designed to assess the effect of LPS on the preference for a palatable solution using a two-bottle paradigm in which mice could choose between a bottle of water and a bottle containing a sucrose solution. The fluid intake was measured by weighing water and sucrose bottles at 2 and 24 h. Another set of mice ($n = 10$ animals per group) received injections of dipyrone or saline 30 min prior to LPS (1000 μg/kg) both administered at 12:00 pm. At 2:00 pm, mice had access to water and 10% sucrose for 24 h. Fluid intake was measured by weighing the water and sucrose bottles at 2 and 24 h. Sucrose preference was determined using the following equation: sucrose intake/total fluid intake (water + sucrose intake) × 100 [18].

2.4. Food intake

Mice were weighed and assigned arbitrarily to body weight-matched groups and were deprived of food for 24 h but still had free access to tap water. On the following day, the animals received injections of dipyrone ($n = 6$ animals) or saline (i.p., $n = 13$ animals) and 30 min later (around to 12:00 pm) were administered an injection of LPS (100 μg/kg, i.p.) or saline (i.p). Immediately after, pre-weighed chow pellets were offered to the animals. Food intake was measured at 2, 4, 6 and 24 h by weighing the remaining food pellets along with any spillage into the cage.

2.5. Statistical analysis

The results are reported as the mean ± S.E.M. Analysis of variance (ANOVA) followed by the Newman–Keuls test was used for comparisons. Differences were considered significant at $p < 0.05$.

3. Results

There was an increase in the immobility period 120 min after the administration of LPS in the FST ($F_{4,39} = 27.3$; $p < 0.001$; Fig. 1A) and TST ($F_{4,39} = 19.4$; $p < 0.001$; Fig. 1B) in mice pre-treated with vehicle. Pre-treatment with dipyrone significantly reversed the LPS-mediated increase in the immobility period in both the FST (at doses of 10, 50 and 200 mg/kg) and TST (at doses of 50 and 200 mg/kg). Furthermore, there was a significant reduction in the number of transitions between the light and dark compartments after LPS administration. The pretreatment with dipyrone (50 and 200 mg/kg) prior to LPS administration caused a greater number of transitions between the compartments compared to the vehicle plus LPS group ($F_{4,39} = 89.6$; $p < 0.001$; Fig. 1C). In addition, LPS significantly decreased the number of line crossings in the center and periphery as well as the total number of line crossings. *Post hoc* analyses indicated that pre-treatment with dipyrone significantly reversed LPS-induced decreases in the number of central ($F_{4,39} = 129.7$; $p < 0.001$; Fig. 1D) and peripheral line crossings ($F_{4,39} = 143.2$; $p < 0.001$; Fig. 1E) as well as the total number of line crossings ($F_{4,39} = 224.5$; $p < 0.001$; Fig. 1F).

The 1000 μg/kg dose of LPS reduced sucrose preference at 2 h ($F_{3,35} = 3.44$; $p < 0.05$) and 24 h ($F_{3,35} = 6.71$; $p < 0.0012$) when compared to the control group (Fig. 2). After 2 h ($F_{4,55} = 2.68$; $p < 0.041$) and 24 h ($F_{4,55} = 7.49$; $p < 0.001$), the dipyrone plus LPS group reestablished the sucrose preference as observed in the control group (Fig. 3).

Compared to the control group, LPS significantly depressed food intake 2, 4, and 6 h after injection as well as overnight food intake (Fig. 4). Pre-treatment with dipyrone (at doses of 10, 50 and 200 mg/kg) significantly reversed the anorexic effect induced by LPS at 2 h ($F_{4,39} = 7.3$; $p < 0.002$), 4 h ($F_{4,39} = 13.84$; $p < 0.001$), 6 h ($F_{4,39} = 13.89$; $p < 0.001$) and 24 h ($F_{4,39} = 7.17$; $p < 0.001$).

The animals that received dipyrone plus vehicle (saline), regardless of dose, showed no significant differences in food intake or sucrose preference in relation to the control group (data not shown).

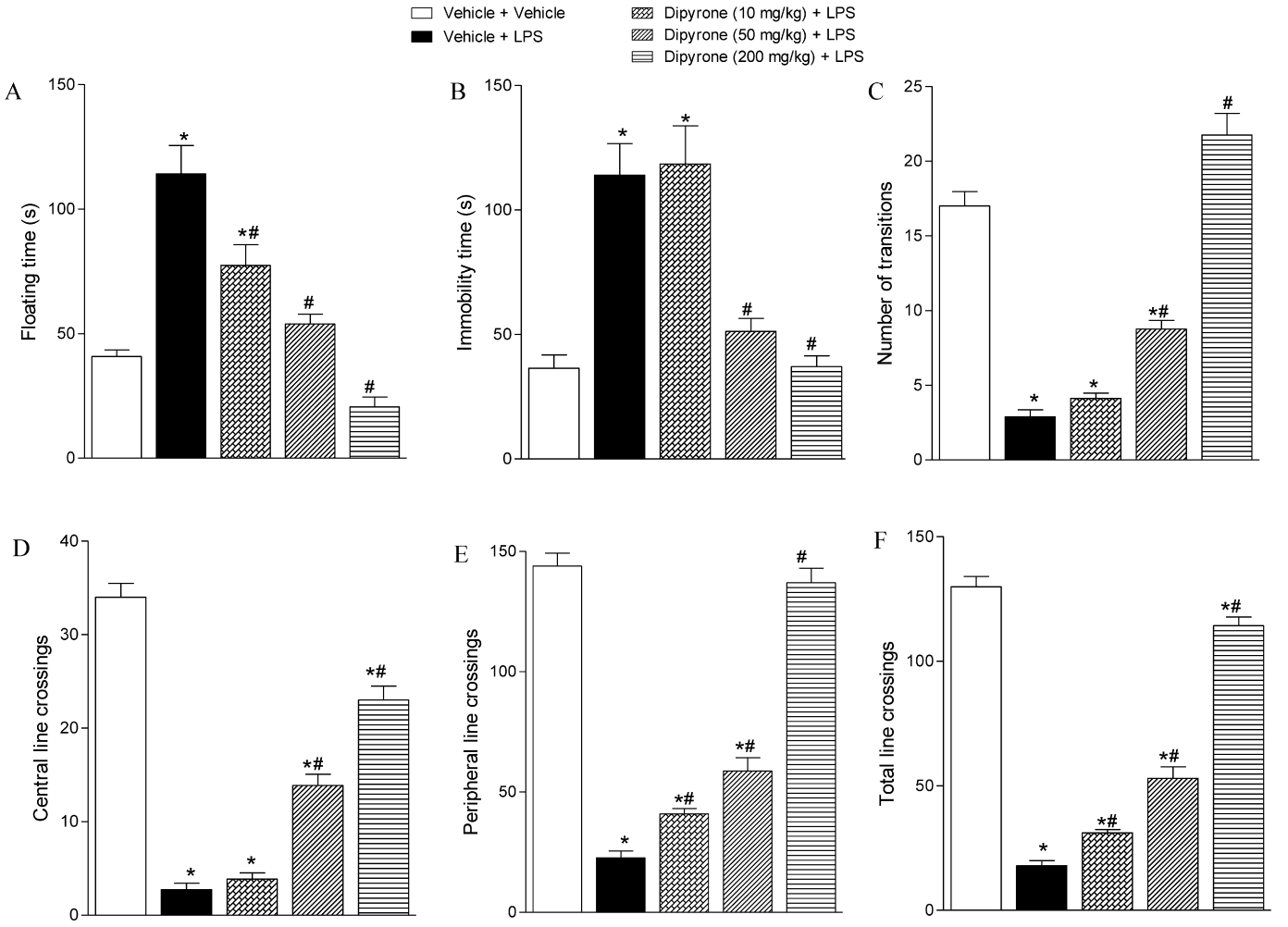

Fig. 1. Effects of pre-treatment with dipyrone (10, 50 or 200 mg/kg) or vehicle associated with the administration of either lipopolysaccharide (LPS) or saline on behavioral tests. Floating time in the forced swim test (FST; A), immobility time in the tail suspension test (TST; B), number of transitions in the light–dark box test (C); central (D), peripheral (E) and total (F) line crossings in the open field test in mice. Results are shown as the mean ± S.E.M. The symbols denote significance levels: $^{*}p < 0.05$ when compared with control groups and $^{\#}p < 0.05$ when compared with the vehicle + LPS group.

4. Discussion

The present results show that dipyrone administered prior to lipopolysaccharide (LPS) attenuates exploratory behavior and locomotor activity and the anorexia and anhedonia caused by LPS administration, suggesting a possible involvement of cyclooxygenase and subsequent release of prostaglandin E2 (PGE2) in sickness behavior.

Reduction in food intake is one of the hallmark symptoms observed after activation of the immune system. Injection of LPS or cytokines results in decreases in food intake [5,20]. Direct action via central signaling mechanisms is better substantiated for the role of pro-inflammatory cytokines in illness anorexia than peripheral action on afferent nerves [1]. Cytokines may act directly on neuronal receptors, either through active transport across the blood–brain barrier [2] or after passive diffusion into the circumventricular organs [12]. However, further evidence has indicated that non-neural cells of the blood–brain barrier, i.e., endothelial cells and perivascular cells such as astrocytes and microglia, are the most important sites of LPS and cytokine action in illness anorexia mediated by prostaglandins [1,9,23]. Several lines of evidence indicate that PGE2 potently inhibits eating [1,19,20], and

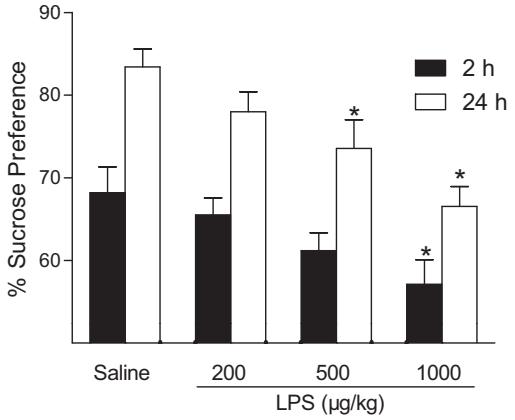

Fig. 2. Preference for 10% sucrose solution 2 and 24 h after lipopolysaccharide (LPS; 200, 500 or 1000 μg/kg) or vehicle administration in mice. Results are shown as the mean ± S.E.M. The symbol denotes significance level: $^{*}p < 0.05$ when compared with the saline group.

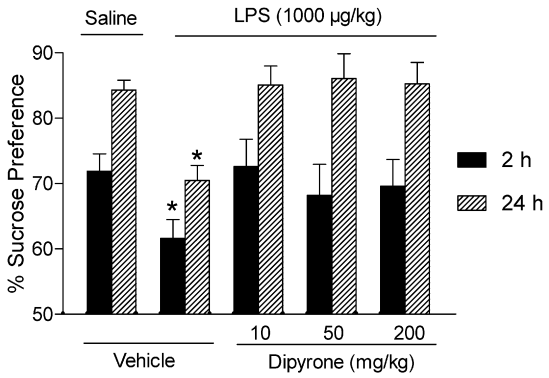

Fig. 3. Effects of pre-treatment with dipyrone (10, 50 or 200 mg/kg) or vehicle associated with the administration of either lipopolysaccharide (LPS) or saline on preference for 10% sucrose solution. Results are shown as the mean ± S.E.M. The symbol denotes significance level: $^{*}p < 0.05$ when compared with the saline group.

injection of LPS or IL-β increases COX-2 and mPGES-1 expression in brain endothelial cells [24]. Considering the present results, inhibition of PGE production by dipyrone is a compelling argument for explaining the reversal of the LPS effect on food intake. In addition, the anorectic response to IL-1 has been shown to be absent or greatly attenuated in mice with a deletion of the *Ptges* gene, which encodes the microsomal prostaglandin E synthase (mPGES). These results suggest that IL-1-induced anorexia is mainly prostaglandin-dependent and is most likely mediated by a mechanism similar to the one that is critical for febrile responses [6,11].

The present study confirms previous observations that LPS can induce depressive-like behavior and a reduction in exploratory behavior in mice [9,10,30]. The doses the LPS change with the experimental models to distinguish and highlight the effects of LPS in each experimental model. It also shows that these effects can be reversed by pre-treatment with dipyrone. LPS administration increased floating time in the FST, increased immobility time in the TST, and depressed locomotor activity in the open field test. The light–dark test is based on a natural situation where an animal is exposed to an unfamiliar environment. This test utilizes the

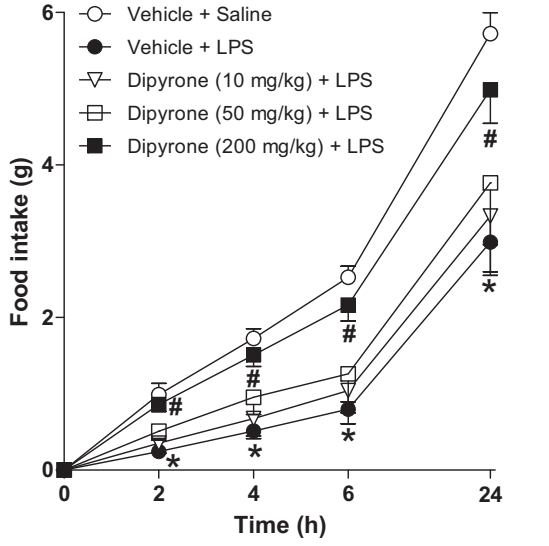

Fig. 4. Effects of pre-treatment with dipyrone (10, 50 or 200 mg/kg) or vehicle associated with the administration of either lipopolysaccharide (LPS) or saline on food intake in mice. Results are shown as the mean ± S.E.M. The symbols denote significance levels: $^{*}p < 0.05$ when compared with control groups and $^{\#}p < 0.05$ when compared with the vehicle + LPS group.

animal's innate preference for the dark and avoidance of bright spaces to assess anxiety-related behaviors [3]. In addition to illness, LPS elicited an anxiogenic-like response in the light–dark box test, characterized by a decrease in the number of transitions between the dark and light compartments of the apparatus [10]. Teeling et al. previously related the behavioral changes induced by LPS in the burrowing and open field tests to PGE2 in the brain [30]. Furthermore, de Paiva et al. provided evidence that prostaglandin synthesis is necessary for the development of depressive-like and exploratory behavior in mice as COX inhibitors also abolished this response and decreased LPS-induced behaviors [9].

In addition to its ability to increase the duration of immobility in the forced swim test and the tail suspension test and to reduce exploration in the light–dark box and open field tests, LPS also depressed sucrose consumption in the sucrose preference test. Blunted sucrose intake in this test has been proposed to reflect impaired sensitivity to reward and to model anhedonia, a core symptom of major depression [8]. The present data demonstrate that the LPS-induced anhedonic behavior was reversed by treatment with dipyrone. Injections of LPS or the individual cytokines themselves resulted in a reduction in food intake and a reduction in the intake of palatable substances such as sucrose [5] and saccharin [8]. In the present work, we examined the anhedonic behavior in mice using different doses of LPS and a 10% sucrose solution. For all LPS doses tested, we observed an interaction between inflammation and sickness behavior.

The importance of COX in depressive pathology is highlighted by recent findings demonstrating that the inflammatory enzyme mediates many of the central effects of psychologically relevant stressors [21,22] and that peripheral application of LPS serves as a model for major depression [26]. It has been reported that chronic treatment with celecoxib, a selective COX-2 inhibitor, reverses chronic, unpredictable stress-induced depressive-like behavior by reducing COX-2 expression in the rat brain [14]. COX-2-selective NSAIDs, developed to reduce the burden of gastrointestinal toxicity, raise concerns about cardiovascular safety through an unknown mechanism [13]. On the other hand, non-selective COX-inhibitors are anti-inflammatory, analgesic and also cause gastrointestinal toxicity. Furthermore, Hinz et al. [15] showed that the major metabolite of dipyrone, MAA, elicits a pronounced inhibition of both COX-1 and COX-2 in vitro and ex vivo in the blood of patients treated with clinically recommended dipyrone doses. After oral administration of dipyrone, rapid occurrence of relevant MAA plasma levels as early as 15 min post administration, inhibition of either isoform showed a virtually instantaneous onset even. Dipyrone, in contrast to classical COX-inhibitors, has a low gastrointestinal toxicity, indicating a different mode of action [25]. Dipyrone has been postulated to act centrally through the inhibition of COX-3, a variant of COX-1; however, the mechanism of action of pyrazolone drugs is unknown [2,27]. Previous studies have shown COX-3 to be sensitive to drugs that are analgesic/antipyretic but which have low anti-inflammatory activity. Dipyrone inhibits COX-3 with an IC_{50} value 6-fold lower than COX-1 but shows no detectable inhibition of COX-2 [4].

Dipyrone has been blamed for causing agranulocytosis. Although it appears to be a statistically significant effect, the incidence is extremely low (1 case per million treatment periods) [15,16]. However, more clinical investigation is necessary with dipyrone. In animals, studies suggesting a minor anti-inflammatory action of dipyrone compared with NSAIDs [29] have yet to be addressed in humans [15]. Dipyrone is a drug widely used in Latin America, Germany and several European countries. Preclinical reports have shown that dipyrone acutely enhances morphine-induced antinociception [28].

In summary, pre-treatment with dipyrone attenuated behavioral changes as well as anorexia and anhedonia in mice challenged

with LPS, suggesting that LPS-induced sickness behavior is dependent on the COX pathway.

Authors' contributions

The work presented here was carried out in collaboration with all authors. AGP (PhD) and RS (PhD) defined the research theme, designed methods and experiments, interpreted and presented the data and wrote the paper. CAFA (PhD) contributed to the anhedonia test protocol and interpretation of all data. DFS, APN and DSB (students) carried out the laboratory experiments and analyzed the data. All authors read and approved the final manuscript.

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